Metabolome analysis of saliva

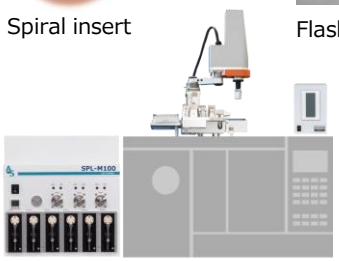
by online solid-phase analytical derivatization GC-MS system

Introduction

Solid-phase derivatization (SPD) is a technique of derivatization without the time-consuming centrifugal concentration and lyophylization, by retaining the target compounds on a solid phase and dewatering it by passing a organic solvent through it then infiltrating the derivatization reagent and performing the reaction on the solid phase. The example of pretreatment method and analytical condition for metabolome analysis of saliva are shown below.

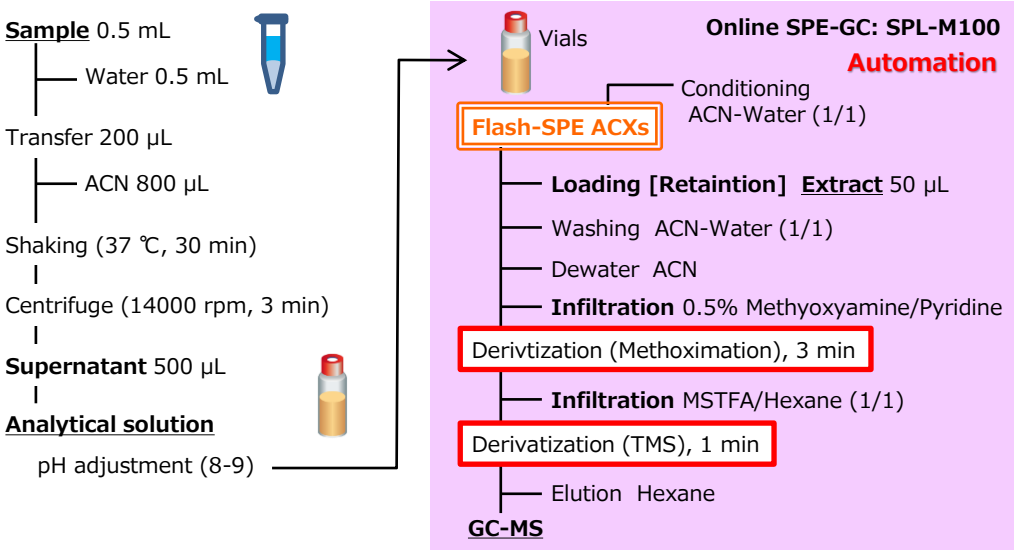
Spiral insert

Flash-SPE



Online SPE-GC-MS system

SPD pretreatment workflow



Analytical condition

SPE-GC interface	**SPL-M100 (AiSTI SCIENCE)**
SPE cartridge	Flash-SPE
PTV injection port	**LVI-S250 (AiSTI SCIENCE)**
Insert type	Spiral insert
Temp.	220℃(0.5 min)-50℃/min-290℃(23 min)
Gas chromatograph	
Inlet mode	Split 1:50
Flow mode	Constant flow, 1.0 mL/min
Pre-column	0.25 mm i.d. x 0.5 m
Column	Vf-5ms, 0.25 mm i.d. x 30 m, df=0.25 µm
Oven Temp.	100℃(2 min)-10℃/min-320℃(2min)
Transfer line Temp.	290℃
Mass spectrometer	
Acquisition mode	Scan (*m/z* 70-600)
Data acquisition	3.0-26 min

SPL-M100
for SPE-GC system

Sample

Information

AiSTI SCIENCE

Product

Online SPE-GC
SPL-M100
Solid-phase cartridge
Flash-SPE
GC large volume injection port
LVI-S250

AiSTI SCIENCE CO.,Ltd.

Tel : +81-73-475-0033
E-mail : as@aisti.co.jp
HP : www.aisti.co.jp

Results

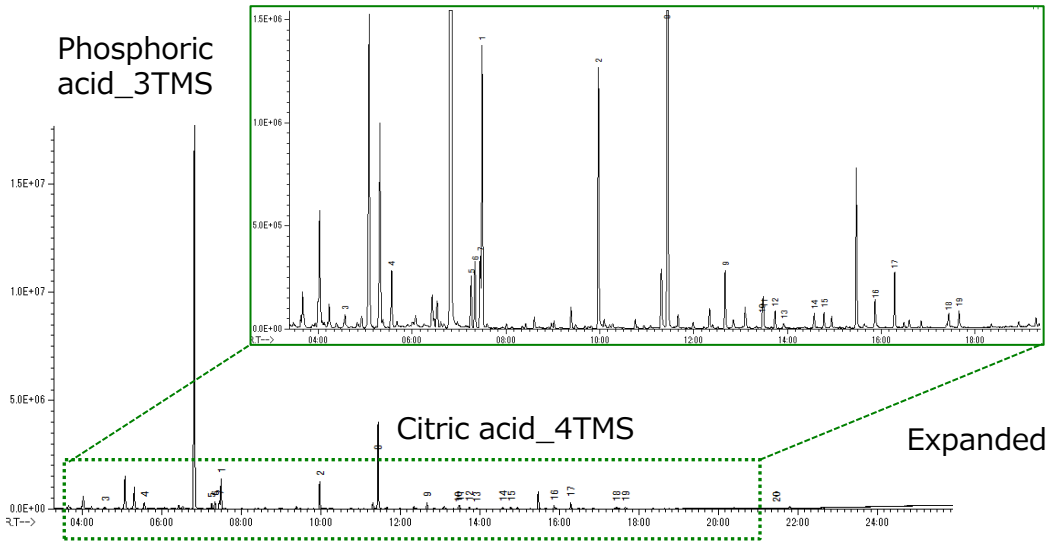


Figure: Total ion current chromatogram

Table: Result of recovery test (n=5)

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Standard solution IS concentrations: 20 μM in vials	S1	2,448,000	111,000
	S2	2,374,000	110,200
	S3	2,313,000	102,700
	S4	2,383,000	110,300
	S5	2,349,000	103,400
	Ave.	*2,373,400*	*107,520*
	RSD, %	***2.1***	***3.8***

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Saliva Dilution: 10 times Spike period of IS: After deprotenization, 20 μM in vials	Feces_K1	2,310,000	126,700
	Feces_K2	2,025,000	115,300
	Feces_K3	2,134,000	117,700
	Feces_K4	2,063,000	109,300
	Feces_K5	2,197,000	120,900
	Ave.	*2,145,800*	*117,980*
	RSD, %	***5.3***	***5.5***
(K/Sx100)	Recovery, %	**90**	**110**
Saliva Dilution: 10 times Spike period of IS: Before extraction, 200 μM in saliva (20 μM in vials)	Feces_A1	1,978,000	121,700
	Feces_A2	1,961,000	113,900
	Feces_A3	2,080,000	122,200
	Feces_A4	1,968,000	111,400
	Feces_A5	2,054,000	122,300
	Ave.	*2,008,200*	*118,300*
	RSD, %	***2.7***	***4.4***
(A/Sx100)	Recovery, %	**85**	**110**
(A/Kx100)	Recovery, %	**94**	**100**

Table: Result of repeatability test (RSD%)

No.	Metabolites	1	2	3	4	5	*Ave.*	RSD, %
1	Alanine_2TMS	124,100	108,500	112,100	113,300	114,200	*114,440*	**5.1**
2	Urea_3TMS	59,950	53,270	65,700	54,050	70,000	*60,594*	**12.0**
3	Proline_2TMS	481,000	418,600	437,000	432,700	454,600	*444,780*	**5.4**
4	Glycine_3TMS	354,300	319,700	323,500	322,100	340,900	*332,100*	**4.5**
5	Succinic acid_2TMS	49,020	46,330	45,910	44,520	46,790	*46,514*	**3.5**
6	5-Aminovaleric acid_3TMS	4,982,000	4,212,000	4,519,000	4,623,000	4,786,000	*4,624,400*	**6.3**
7	Putrescine_4TMS	457,300	404,100	422,900	413,200	438,100	*427,120*	**4.9**
8	Citric acid_4TMS	9,392	8,965	8,049	8,148	7,767	*8,464*	**8.1**
9	Ornithine_4TMS	74,060	62,220	63,890	63,740	68,900	*66,562*	**7.3**
10	Cadaverine_4TMS	131,400	113,400	119,100	116,700	124,200	*120,960*	**5.8**
11	Myristic acid_TMS	15,190	14,160	14,430	12,530	14,170	*14,096*	**6.9**
12	Lysine_4TMS	15,050	12,850	13,010	13,890	14,080	*13,776*	**6.5**
13	Tyrosine_3TMS	105,300	90,520	96,260	91,420	96,840	*96,068*	**6.1**
14	Palmitic acid_TMS	73,910	72,730	74,010	63,600	75,890	*72,028*	**6.7**
15	Uritic acid_4MS	122,400	105,400	111,000	111,300	108,700	*111,760*	**5.7**
16	Oleic Acid_TMS	17,690	18,570	16,440	15,330	16,250	*16,856*	**7.6**
17	Stearic acid_TMS	40,930	39,760	38,730	34,410	39,650	*38,696*	**6.5**